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The wound healing property of encapsulated silver nanoparticles made from aqueous extracts of *Elaeis guineensis* Jacq leaves

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Abstract
A wound can be defined as the disruption of the normal integrity of the body. the management of wounds usually ends up with scars that can cause functional harm and emotional and social distress. All these urge the need for new approaches for faster healing by anti-infective effect, moisturizing, stimulating the healing mechanisms, speeding up the wound closure and dropping scar formation, this paper aimed at evaluating the wound healing effect of cellulose nano encapsulated *Elaeis guineensis* silver nanoparticle.
The plants were collected and formally identified at the national herbarium. After drying and grounding, the aqueous decoction was prepared. The phytochemical screening was performed on the crude extract. From the prepared extracts, the nanoparticle was prepared according to the previously described protocol. The prepared nanoparticles were encapsulated with cellulose. The cellule nanocapsules of *Elaeis guineensis* were tested for the *in vitro* anti-inflammatory effect using the heated BSA denaturation test. The acute dermal toxicity of the cellulose nano-encapsulated Elaeis *guineesis* silver nanoparticle was performed according to guideline 402 of the OEDC. The wound healing test was performed on rat dorsal open wounds using a series of doses of the prepared cellulose nano-encapsulated *Elaeis guineesis* silver nanoparticle (CNC-EgNP).
The extract was revealed to contain alkaloids and polyphenols. Heterosids were not detected. The nanoparticle was successfully prepared, and nano encapsulated with cellulose was successfully done when referring to the absorption feature of the mixture. The results showed that CNC-EgNP protects the protein from heat-denaturation with up to 94.9% with 25µg/ml. Compared to diclofenac, used here as a reference, at 200 µg/ml, the CNC-EgNP seems more effective with 54,2% and 98,65% respectively. No sign of toxicity was observed from the toxicity testing. The CNC-EgNP 200mg/kg BW has the best effect as it annuls the wound after 13 days. CNC-EgNP 100 and 400 mg/kg BW and crude extracts had a statistically similar effect. All annul the wound on day 15. Up to that period, the trolamine, used here as a positive control, failed to bring to 0 the area of the wound.
The results highlight the potential of the cellulose nanocapsule of *Elaeis guineensis* silver nanoparticle as an alternative for wound management.

Keywords: *Elaeis guineensis*, silver nanoparticle, cellulose nano-capsule, wound healing, anti-inflammatory

Introduction
A wound can be defined as the disruption of the normal integrity of the body. This can be after physical damage, fissure erosion or ulcer. While erosion (local loss of epidermis without disturbance on dermis) and fissure (tissue loss due to fracture in the epidermis and or dermis) are both rare, the ulcer is more common. The ulcer is a wound located in the dermis with epidermal tissue. It is one of the conditions that usually raises concern for clinicians. The most important situation refers to as wounds are those caused by physical damage to the skin. The wound management outcome depends on a wide and complex range of parameters including the patient's general condition, the cause of the sound and the treatment strategy. These treatment strategies are drug based and are targeting mostly bacteria, fungi and viruses that are colonising the wound. The increased resistance to available antimicrobial drugs, in addition to wound fact that the management generally ends up with scars that can cause functional harm and emotional and social distress [1]. All these urges the need for New approaches for faster healing by anti-infective effect, moisturizing, stimulating the healing mechanisms, speeding up the wound closure and dropping scar formation [2].

Natural compounds from the plant, are broadly used in traditional medicine for the management of disease conditions including wounds. *Elaeis guineensis* Jacq commonly known as the palm oil tree in Cameroon is wide use in traditional medicine for wound healing purposes. In Ghana, powdered leaves are used in chronic wounds, and in Gabon, the juice of the leaves is applied directly to wounds created by skin cuts. Several scientific reports are advocating that the leaves of *Elaeis guineensis* are efficient in healing wounds through their antibacterial effect or anticandidal effect, and more interesting through wound disclosure and tissue regeneration in the broken part of the skin. Ointments have been prepared from *Elaeis guineensis* and successfully demonstrated a healing effect on wounds in the murine model of the wound. The standardisation of the leaves has been done by FTIR, gross morphological, and microscopical descriptions. To the best of our reading, there is no report on the nanoparticle nor nanocapsules from the *Elaeis guineensis* leaves extracts.

The application of nanotechnology to medicine has recently been revolutionizing the management and the healing of wounds with novel approaches. This technology is possibly the next generation strategy for the advancement of wound healing and the treatment of chronic wounds [2]. With respect to that, the possibility of using noble metal nanoparticles for regenerative medicine is considered. Noble metal nanoparticles such as gold, palladium, and platinum nanoparticles due to their strong catalytic capacities are said to be good antioxidants. Silver, gold, platinum, selenium, copper, zinc oxide, tantalum oxide, iron oxide, and titanium dioxide nanoparticles have shown potential therapeutic effects. The most reported nanoparticle is that made of silver (Ag) for wound healing [3]. Material derived nanoparticle in terms of metal nanocomposite [3] and the ability they are offering for delivering active principle to the desired tissue to produce the healing effect are increasingly being used in medicine [3]. This broad use of silver nanoparticles will further increase the risk of human health resulting from repeated exposure from different sources and inefficient delivery. Therefore, silver nanoparticle delivery strategy is of high interest [5]. Nanocapsules can close the active ingredients including nanoparticle in their inner part and release them in the desired time frame. These systems are used for the cargo of biologically active substances. The ability to deliver active ingredients to the right place and their controlled release are very important features ensured by nanoparticles [5–12]. The encapsulation system are said to maximized benefits of the nanoparticle based on natural compounds. Moreover, This protect sensitive natural compounds and unstable nanoparticle from oxidation, dehydration [12]. Many polymers have been used for that end. These include Gelatine, acacia gum, chitosan [12], poly(isobutylcyanoacrylate) [12, 13] dipalmitoyl-phosphatidyl choline (DPPC) and cholesterol [5], Span® 80/poly(ε-caprolactone) [14], DL-α-tocopherol Tween® 80 [15], Maisine™ 35-1/Epikuron® 145V [15], cellulose nanocrystals (CNCs)—nanomaterials [16, 17]. To the best of our reading, there is no report on the encapsulation of silver nanoparticle of leaves of *Elaeis guineensis* Jacq. This article aimed to evaluate the wound healing and anti-inflammatory effect of the encapsulated silver nanoparticle of the aqueous extract of *Elaeis guineensis* Jacq.

Material and Method

Plants material

The leaves of *Elaeis guineensis* were collected in the Douala Littoral region of Cameroon and brought to the Laboratory. A voucher specimen was deposited at the National herbarium under the identification number 34163HNC.

Animal material

The Animal material was composed of Wistar rats.

Elaeis guineensis extract preparation

The extraction preparation was performed according to the protocol described by Eya'ane Meva *et al*., [18]. Briefly. After collection, healthy leaves were cut from the branch and washed under flowing water to remove trace of dust, epiphytes, and pesticide residues. Then they were shopped in piece, and 50g were weighted and infused in 500ml and the temperature was maintained at 80 °C for 5 more minutes and the filtrate throughout Whatman N°1 paper. After cooling, the filtrate was stored at 4 °C for no longer than one week, prior to the test.

Phytochemical Screening

Phytochemical screening tests were performed on the filtrate following standard procedures as previously reported earlier [19–21].

Synthesis of silver nanoparticles (AgNP)

The silver nanoparticles were prepared as previously describe by Eya'ane Meva *et al*., [22]. Briefly, to 50 ml of $AgNO_3$ (10-3M) 10 ml of filtrate were added. The mixture was homogenised for 1min and store in dark at room temperature for reaction to take place in static conditions. All the water used in this part was deionised water and all glass material used as containers were throughout washed with dilute nitric acid and rinsed many times with deionised water.

Nano cellulose preparation by the acid hydrolysis method (CNC)

The nanocellulose was prepared according to the general acid hydrolysis method described previously by Onkarapa *et al*., [23]. Briefly, cellulose (5g) was dissolved in 50ml of 60% sulfuric acid. The preparation was allowed to react at 45°C under 500rpm continuous stirring for 1 hour. The hydrolysis was reduced by adding 100ml of 4°C distilled water. The obtained paste was washed with water by centrifugation with pH monitoring. The last wash was performed with 2%NaOH until pH7. The suspension obtained was then disperser under 10 min of sonication to obtain the nanocellulose crystals. The solution was stored at 4°C prior to further preparation.

Synthesis of encapsulated nanoparticle

The cellulose nanoencapsulation of organic silver nanoparticle was perfume according to the protocol previously describe by Li *et al*., [24]. Briefly, 0.17g of EgNP were added to 50ml of the solution CNC keep in water bath at 80°C. Then the pH of the solution was brought to 8 with addition of NaOH. Subsequently, 2g of pure glucose was added to the mixture and the mixture for 4 hours. The obtained solution was gradually washed under centrifugation with methanol and water for 30 minutes to obtain the desired CNC-EgNP.

The yield of encapsulation was estimated using the following formula

$$Yield = \frac{\text{(CNC−EgNP) weight}}{\text{weight on EgNP}} \times 100$$

Evaluation of acute dermal toxicity

Nanoparticle innocuity testing was performed according to OEDC guideline 402 of the OEDC [25]. Briefly, young nulliparous female rats aged between 8 and 12 weeks are

provided by the animal house of the Faculty of Medicine and Pharmaceutical Sciences of the University of Douala-Cameroon, where they are housed and fed with strict respect with the ethical rules of having food and drinking water *ad libidum*. Having natural light 12 hours light, 12 hours dark with natural temperature as in town.

Prior to tests, 6 females of the same bird batch were randomly selected according to their weight (160-200g) and acclimatized in the testing room for a week under the same condition as in the growing room. 24 hours before the tests, the dorsal/flank area of the test animals were closely removed attention being paid to avoid epidermis alteration. On the testing day, 2000mg/kg BW of the CNC-EgNP was uniformly applied to the shaved area and the treated area was protected with sterile bandage, to protect the animal from eating, and maintained for 24 hours. For addition caution, each animal was individually place in cages. The animals were observed immediately after CNC-EgNP application during the first 30 minutes, each hour to up to 6 hours then, periodically during the first 24 hours, and daily thereafter, for 14 days total. All signs or animal behaviour were recorded as recommended by the OEDC.

In vitro evaluation of the anti-inflammatory effect of the CNC-EgNP

The anti-inflammatory effect of the cellulose encapsulated silver Nanoparticle of *Elaeis guineense* (CNC-EgNP) was assessed using the inhibition of heat denaturation of Bovin serum albumin model as previously described [26, 27] with slight modification. Briefly, to 2,8ml of PBS, 0,2ml of BSA (5% in PBS), and 2 ml of different concentration (25, 50, 100, and 200 µg /ml) of CNC-EgNP or diclofenac were mixed and preincubated at 37 °C in incubator for 3 hours then the temperature was increase to 70 °C for 5 mins. The mixture was cooled down and the volume was completed with 2.5ml of PBS. The absorbance was measured at 660nm using PBS as a blank. The anti-inflammatory activity was estimated in term of percentage inhibition of protein denaturation and calculated by using the following equation:

$$\%I = \frac{(Abs\ control - abs\ test)}{\text{Abs control}} \times 100$$

Evaluation of wound healing activity

The wound healing effect of the CNC-EgNP was assessed using the protocols previously described in the literature [19, 27, 28].

Animal and ethical considerations: 24 healthy adult rats randomly selected from the same bird batch at the FMPS animal house and housed in the same condition of temperature, food, and water. The protocol was approved by the ethical committee of the University of Douala (protocol number 3156CEI-UDo/06/2022/T).

Excision wound model: After a one-week acclimatization in the testing room, the animals were weighted, marked, grouped according to their weight into six groups and anesthetized using the murine anesthesia protocol number ANE-06 by intraperitoneal of a solution made of Kétamine/Diazépam (50mg/ml)/ (10mg/2ml). After sterilisation with 70% ethanol, at 1 cm away from vertebral column, the fur from the dorsal region was shaved aiming at outlining the area of the wound to be created. The wounds were created along the markings (2.5 cm x 2.5 cm) region of the skin using toothed forceps, scalpel, and scissors created by aseptically cutting a piece of 6.25cm^2 (2.5 cm x 2.5 cm) skin which was left opened. Haemostasis was achieved by blotting the wound with a cotton swab soaked in normal saline.

Animal grouping and treatment

The wounded animals were place in individual cages and groups in to six different groups. The group 1 was the negative control and received normal saline. Group 2 was the positive control and received trolamine (reference). Group 3 receive the plant extract at 400mg/kg body weight. Groups 4, 5 and 6 receive CNC-EgNP at 100, 200, and 400 mg/ kg body weight, respectively.

Wound healing follow-up

The wound closure rate was assessed by tracing the wound on each two days for twenty days. The wound areas were measured using translucent paper and pencil. The drawn square was measured, and the surface evaluated. The percentage of the wound was estimated using the following equation [28].

$$\%Wound\ closure = \frac{(woundareaon\ 1st\ day - Wound\ area\ on\ day\ (n)}{(woundareaon\ 1st\ day)} \times 100$$

Were *n* is the number of measurements days.

Statistical analysis

The data collected were entered and summarized in Excell and was expressed as mean ± Standard Error of the Mean (SEM). Finally, one-way analysis of variance (ANOVA) followed by post hoc Tukey's test was used and P value < 0.05 was considered statistically significant.

Results and Discussions

Results

Yield of extraction and phytochemical screening of the extract.

From 50g of leaves, 3.54 g of an aqueous soluble smooth paste were obtained, giving a yield of 7.08%. regarding the main phytochemical group present in the extracts, the results. Of the phytochemical screening revealed, that apart from sterols and heterosids that were absent, all other main secondary metabolites were detected (Table 1.)

Table 1: Results of the phytochemical screening of the crude extract of *E. guinnensis*

Chemical groups	results
Alkaloids	+
Tanins	+
Flavonoïds	+
Sterols	-
Cardiac glycosids	-
Anthraquinones	+
Saponines	-

+= present; - absent.

Synthesis and characterization of the EgNP

The synthesis of the nanoparticle was monitored by observation of the colour change between the starting and the ending colours visually (from yellowish to dark brown) and spectrophotometric ally between 350 and 550nm (figure1).

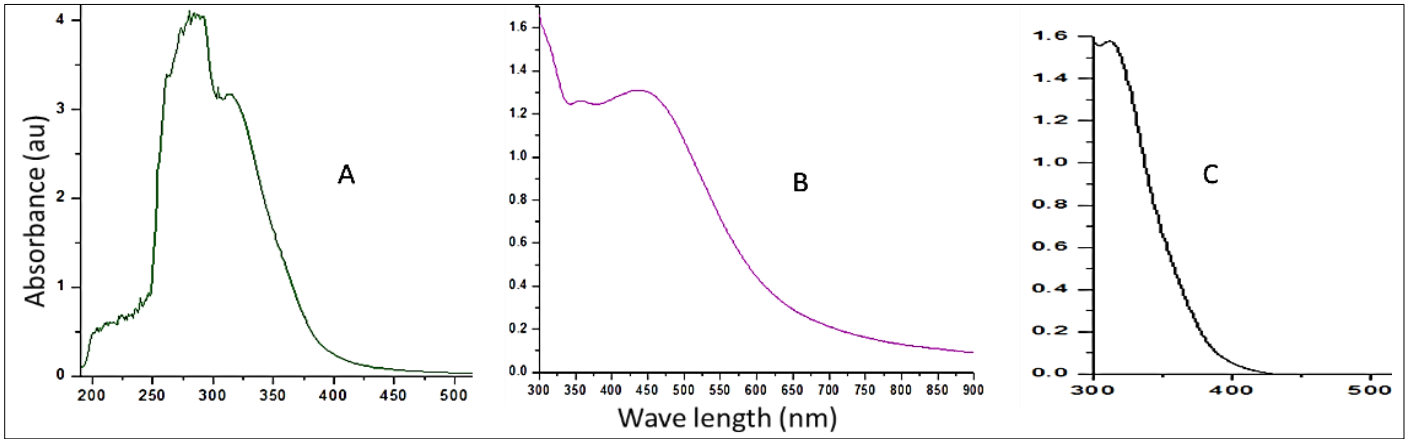


Fig 1: spectrophotometric behavior of the crude extracts (A), the Elaeis guinnensis sylver nanoparticle (B) and the supernathan of the prepared nanoparticle (C).

From figure 1, it came out that the nanoparticle was successfully synthesised, with the disappearance of the compound from the crude extract (Figure 1 C) with the formation of the bell shape curve at the region of 350 to 550 nm.

Synthesis of cellulose nano capsule

As for the nanoparticle, the synthesis was monitored for visual and spectrophotometric behaviour of the solution before and after reactions and the results are presented in Figure 2 bellow.

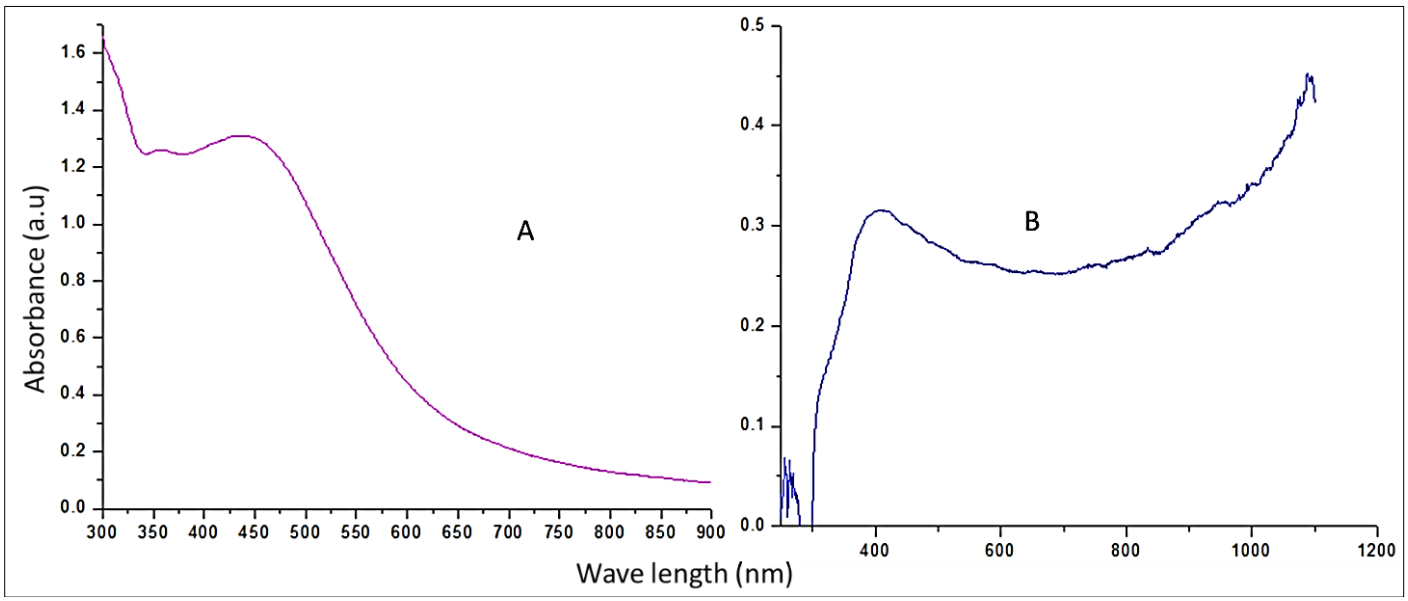


Fig 2: Spectrophotometric behaviour of the *Elaeis guinnesis* silver nanoparticle (A) and the cellulose encapsulated *Elaeis guinnesis* silver nanoparticle

This figure 2 highlights a deep change between the absorbance feature of the EgNP and the CNC-EgNP. These reduction of the absorbance of the mixture lead au to assume that the end product was the encapsulation of the EgNP thereby, reducing his ability to absorb light. The extraction yield was estimated to be 662.5 per cent.

***In vivo* anti-inflammatory effect**

The results of the antiproteolytic effect of the CNC-EgNP are recorded in figure 3 bellow. These results showed that CNC-EgNP protects the protein from heat denaturation with up to 94.9% with 25µg/ml. In comparison to diclofenac, used here as reference, at 200 µg/ml, the CNC-EgNP seems to be more effective with 54, 2% and 98,65% respectively.

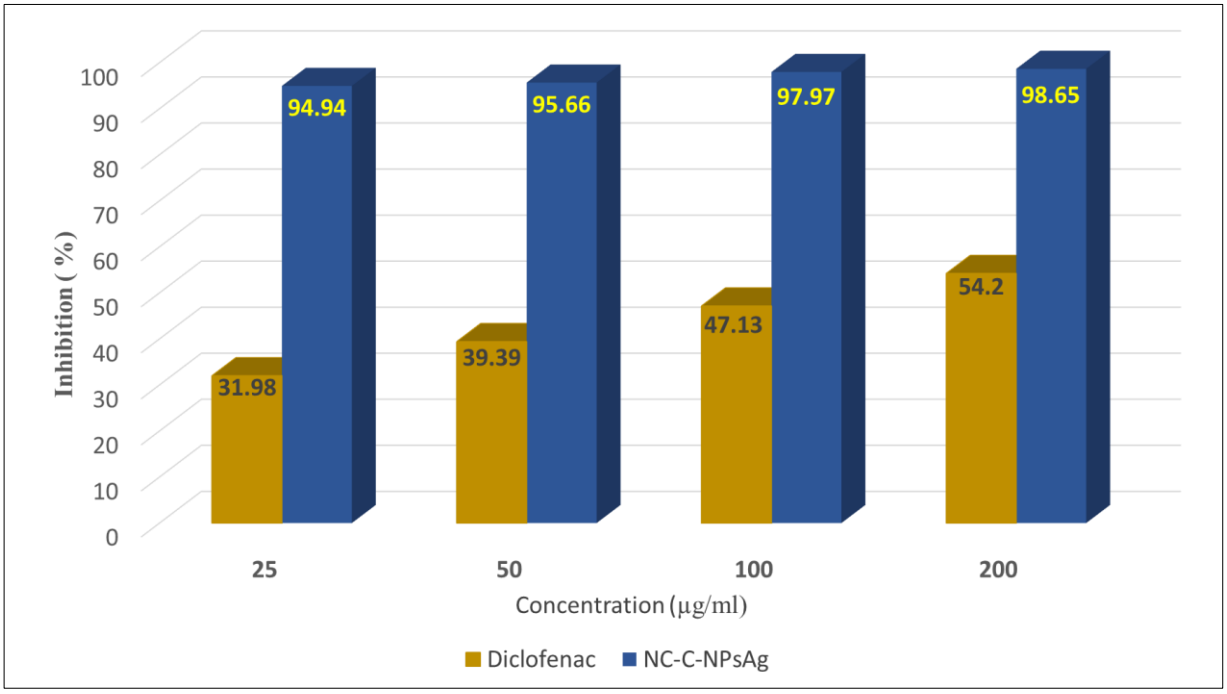


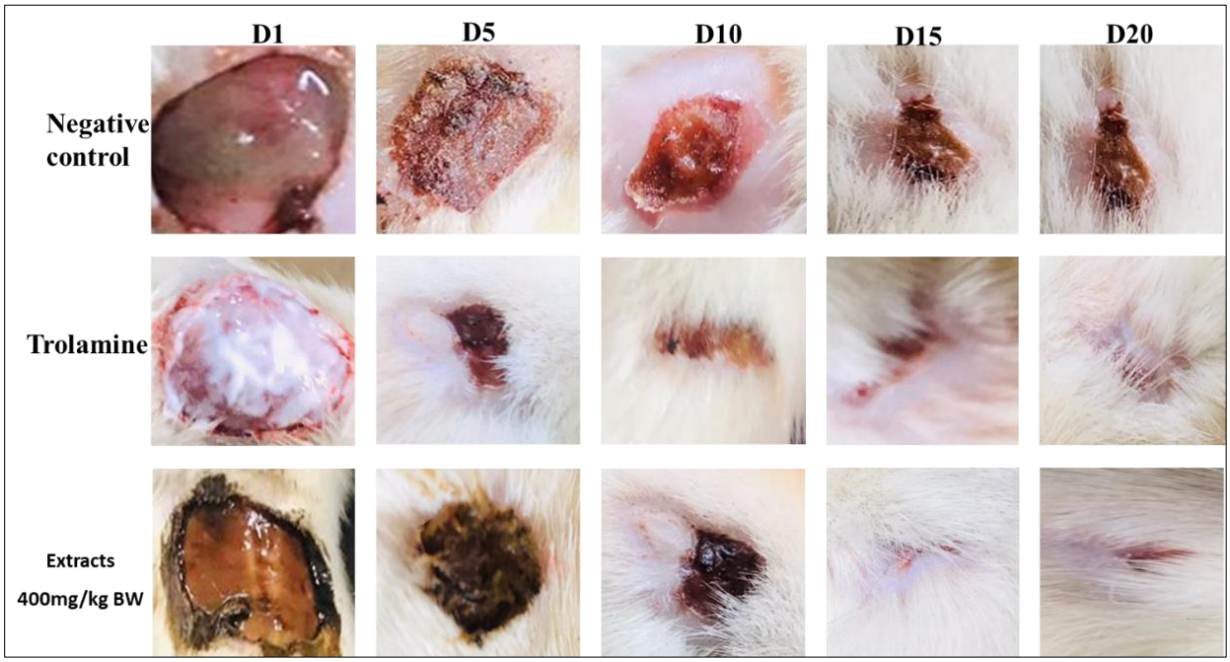
Fig 3: antiproteolytic effect of the cellulose encapsulated *Elaeis guinnesis* silver nanoparticle

Acute toxicity testing

No signs of dermal toxicity were observed.

Wound healing property

The wound healing effect of the CNC-EgNP were assessed over 21 days. The figure 4 showed the picture of the wounds over the time. These pictures show that the model is valid, as there is a significant difference between the reference rat and the untreated rat (figure 4). The feature of the wound treated with different drugs revealed that, with regards to the negative control, the CNC-EgNP at all doses as well as the crude extract of *Elaeis guinnensis* have a better wound healing effect in comparison to trolamine (figure5). The CNC-EgNP 200mg/kg BW have the best effect as, it annuls the wound after 13 days. CNC-EgNP 100 and 400 mg/kg BW and crude extracts had the statistical similar effect. All annul the wound on day 15. Up to that period, the trolamine, used here as positive control, failed to bring to 0 the area of the wound.



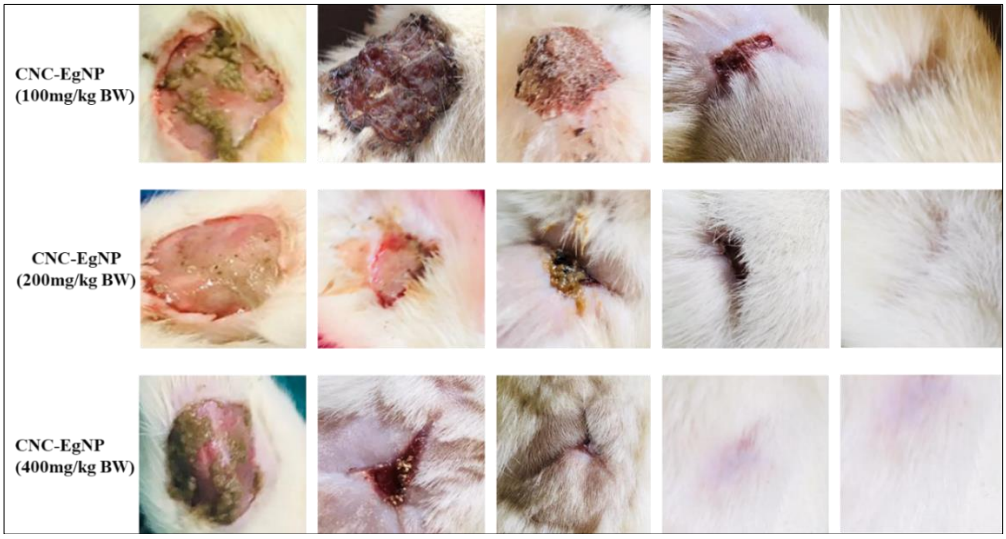
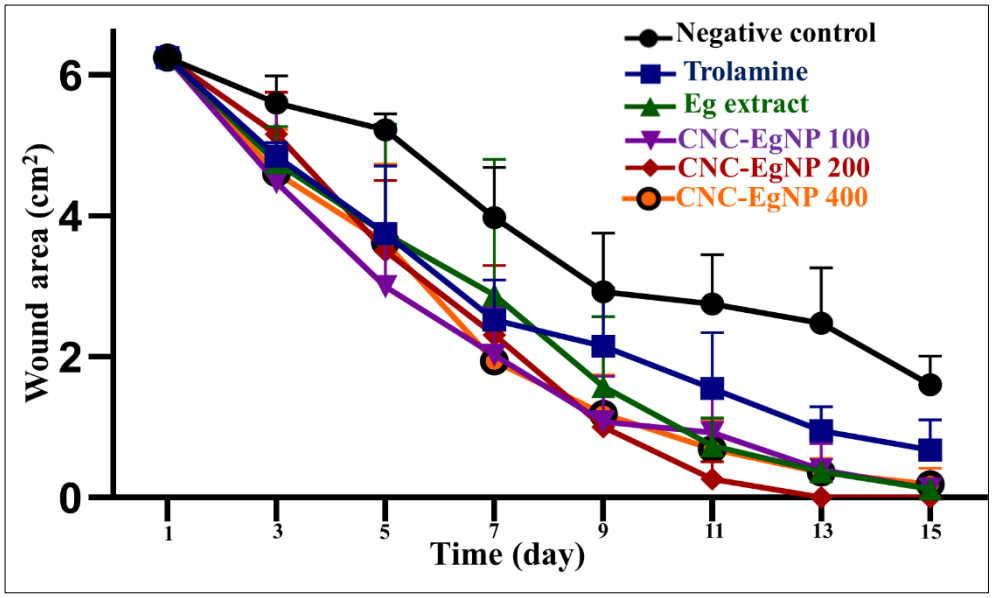


Fig 4: Wound healing effect of the CNC-EgNP, Trolamine, and crude aqueous extracts of *Elaeis guinnesis*.



Eg extract = Elaeis guinnense extract; CNC-EgNP X= cellulose nano capsule of Elaeis guinnensis silver nanoparticle at Xmg/ kg body weight (X=100, 200 or 400)

Fig 5: reduction in wound area in presence of, *Elaeis guinnensi* crude extract, and various concentrations of nano capsules.

Discussion

The aim of this study was to assess the wound healing property of the cellulose nano-encapsulated silver nanoparticle of *Elaeis guinnensis*.

The phytochemical profile of *Elaeis guinnensis*, revealed the presence of alkaloids and polyphenols. Heterosids and terpenoids were not present in the extracts studied in this study. This is different from what have been obtained previously by Sasidharan *et al.*, [30]. In fact, they obtained with the methanolic extract alkaloids, polyphenols, heterosids, and terpenoids. The difference between these two studies can be attributed to the type of solvent used. Also, it is well documented that methanol is the best extractive solvent. It might extract polar and non-polar compounds from the plant material. But with regards to the environmental friend consideration, this solvent was not used. The second level of explanation of this discordance can be attributed to the extraction time. In this study, the maceration time is 5 minutes. This could not have been enough to allow the extraction of partly polar compounds. However, alkaloids and polyphenols are known to be good wound healing potential [30, 31].

Nanoparticles prepared from plant extracts are reported to have a better effect in comparison if the crude extracts [3, 19, 21, 22, 32–35]. Furthermore, The nano-encapsulation is another process that help in improving the pharmacological effect of crude extracts that is increasingly reported in the literature [21]. Moreover, the encapsulation of nano-particle are reported to have better effect in comparison to the nano-particle and nano-capsule taken alone [5, 6, 8-10, 17, 35, 36].

With respect to wound healing potential, nanomaterials are interesting option in wound management [2]. With that respect, the cellulose encapsulated silver nanoparticle of *Elaeis guineensis* were prepared in this study. To the best of our knowledge, we did not find reports on the neither the nano-capsule, nanoparticle nor encapsulated nanoparticle. However, methanolic extracts of *Elaeis guineensis* leaves have been reported to have wound healing effect on an infected wound model in rats [30]. Furthermore, in the study by Rajoo *et al.*, [29], the effect on the infected wound was reported and revealed that the wound healing effect of the methanolic extract were comparable to that of betadine, used in that study as a positive control. In that study, the wound was completely closed after 20 days. All these reports

advocate for the fact that the extracts of *Elaeis guineensis* have wound healing potential. However, all these reported effects are much lower than what was obtained here with encapsulated nanoparticle. In fact, the healing effect of the encapsulated *Elaeis guineensis* silver nanoparticle was obtained 13 days post treatment at the concentration of 200mg/kg body weight.

This paper highlighted the wound healing potential of the cellulose encapsulated *Elaeis guineensis* silver nanoparticle. Thereby, opening a new door in wound healing strategies.

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